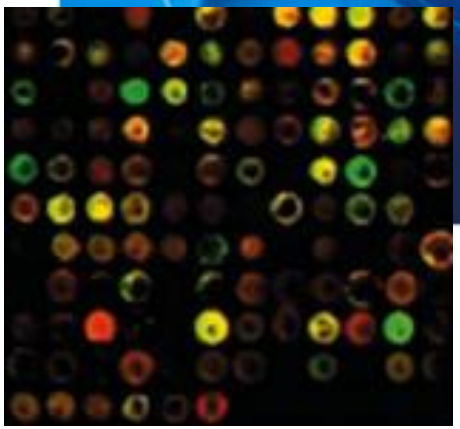
Recombinant DNA and Biotechnology



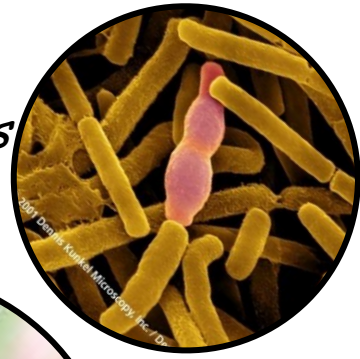
Definitions

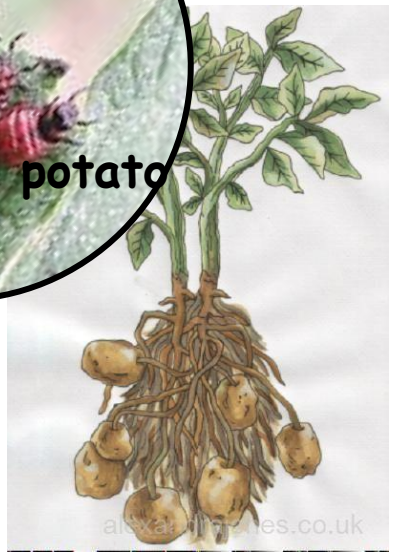
- Recombinant DNA
  - DNA fragments from differet sources, that have been joined together
- Biotechnology
  - The use of living cells for production of e.g. foodstuffs, medicine, chemicals…
- Cloning
  - Producing identical copies of a gene

Example – killer potatoes

- Large amounts of insecticides are used to protect crops
- *B. thuringiensis* is a bacterium that produces a protein toxic to insects
- Transgenic plants (Bt planter) that produce the bacterial toxin are resistant to insect attack

Bacillus thuringiensis





Example – "Golden Rice"

- Vitamin A deficiency causes 6000 deaths every day worldwide –primarily i developing countries.
- The body makes vitamin A from β-carotene obtained via diet
- Rice grains contain only the first part of the biochemical pathway for synthesis of β-carotene

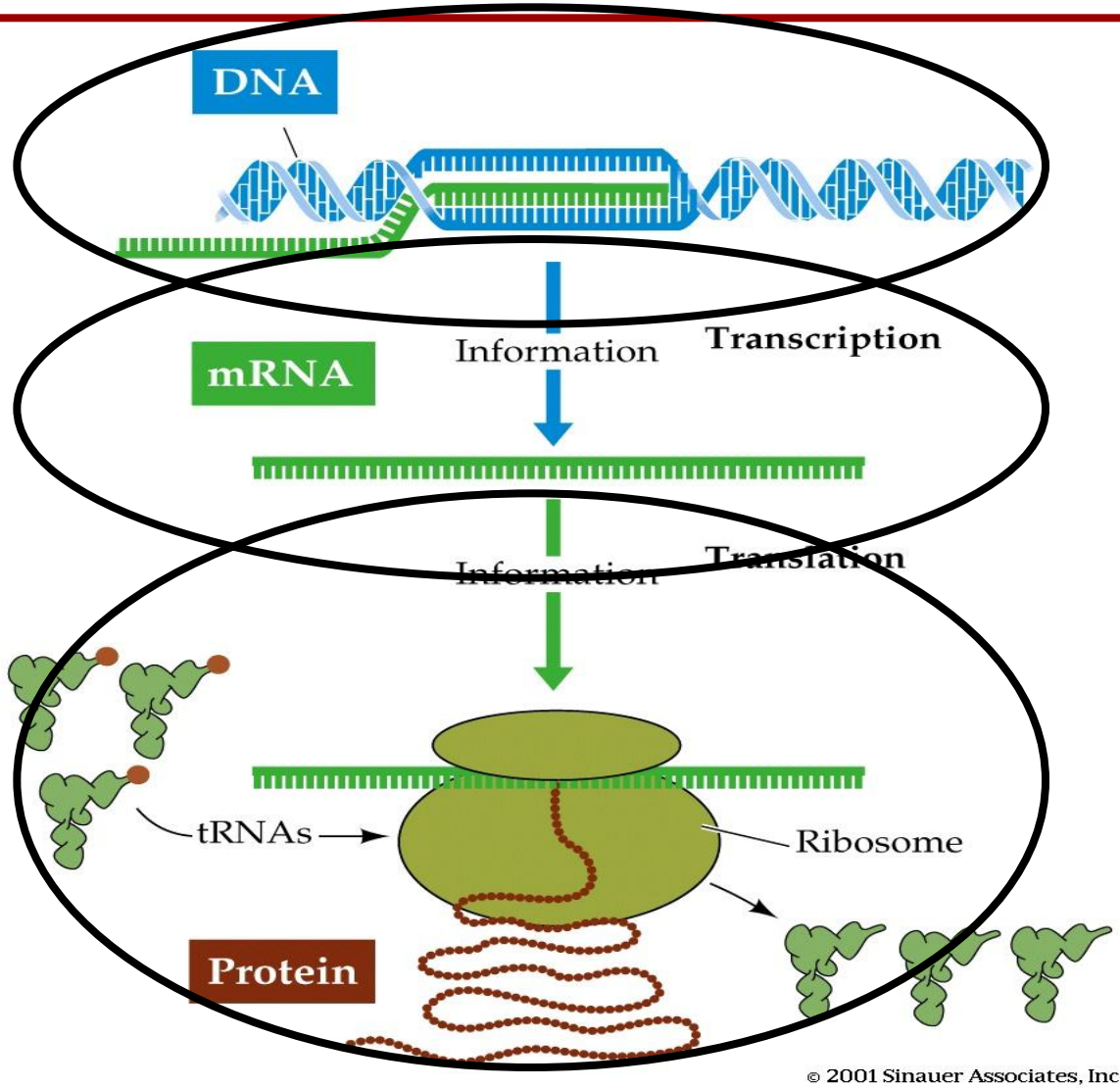
• By adding genes for 2 additional steps i the pathway, rice was developed with high β-carotene levels in the edible part of the grain

Example – "Golden Rice"

- Golden rice grains contain up to 35 µg/g β-carotene in the edible parts (100-150 g can provide 60% of RDA)
- Not possible without GM technology
- Humanitarian project- collaboration between academic researchers and industry (Syngenta)
- Golden Rice was ready i the lab in 1999 but is still not available for farmers due to the comprehensive regulatory procedures for GM crops. Recently 2 field trials were completed in the Philippines.
- Read more: http://www.goldenrice.org

The Central Dogma

Ch 14



The Genetic Code

Ch 14

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phenylalanine UUA, UUG: Leucine	UCU, UCC, UCA, UCG: Serine	UAU, UAC: Tyrosine UAA: Stop codon UAG: Stop codon	UGU, UGC: Cysteine UGA: Stop codon UGG: Tryptophan	U C A G
C	CUU, CUC, CUA, CUG: Leucine	CCU, CCC, CCA, CCG: Proline	CAU, CAC: Histidine CAA, CAG: Glutamine	CGU, CGC, CGA, CGG: Arginine	U C A G
A	AUU, AUC, AUA: Isoleucine AUG: Methionine; start codon	ACU, ACC, ACA, ACG: Threonine	AAU, AAC: Asparagine AAA, AAG: Lysine	AGU, AGC: Serine AGA, AGG: Arginine	U C A G
G	GUU, GUC, GUA, GUG: Valine	GCU, GCC, GCA, GCG: Alanine	GAU, GAC: Aspartic acid GAA, GAG: Glutamic acid	GGU, GGC, GGA, GGG: Glycine	U C A G

From DNA via mRNA to protein (pokaryotes)

Ch 14

DNA: 5′-ATG CCG ATA TAT CCG TAA-3′

3′-TAC GGC TAT ATA GGC ATT-5′

↓ Transcription

mRNA: 5′-AUG CCG AUA UAU CCG UAA-3′

↓ Translation

Protein: Met-Pro-Ile-Tyr-Pro

From DNA via mRNA to protein (Eukaryotes)

Ch 14

Exons

DNA: 5′-ATG CCG GCATGA ATA TAT CCG TAA G-3′

3′-TAC GGC CGTACA TAT ATA GGC ATT C-5′

Introns

Transcription

Pre-mRNA: 5′-AUG CCG GCAUGA AUA UAU CCG UAA GAAAAAA-3

Splicing

mRNA: 5′-AUG CCG AUA UAU CCG UAA GAAAAAA-3

Translation

Poly-A tail

Protein Met-Pro-ILE-Tyr-Pro

Tools for gene technology

- Enzymes to cut and paste DNA
  - Restriction enzymes (Ch 15), DNA ligase (Ch 13)
- Enzymes and method to copy DNA molecules
  - DNA polymerase, Polymerase Chain Reaction (PCR) (Ch 13)
- Ways of getting DNA into cells
  - Transformation, transduction, conjugation, transfection (Ch 12,13,16)
  - Plasmids (Ch 12) and other vectors
- Ways to recognise cells containing recombinant DNA
  - Marker gene encoding e.g. antibiotic resistance

Restriction enzymes – molecular scissors

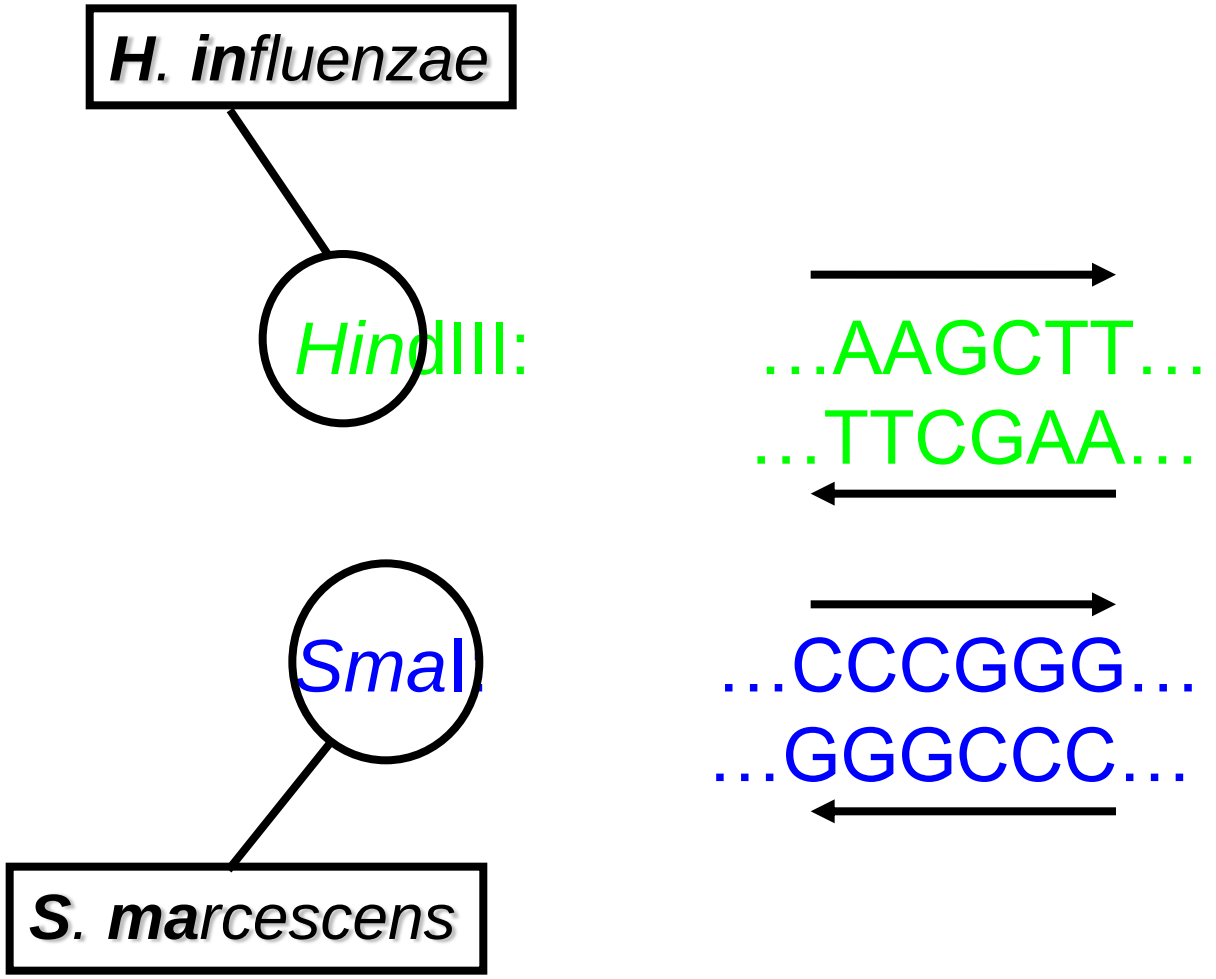
Ch 15

AGAAGCTTTTCACACCCGTTTTTTTTTGATTATCCCGGGCCT
TCTTCGAAAAGTGTGGGCAAAAAAAAACTAATAGGGCCCGGA

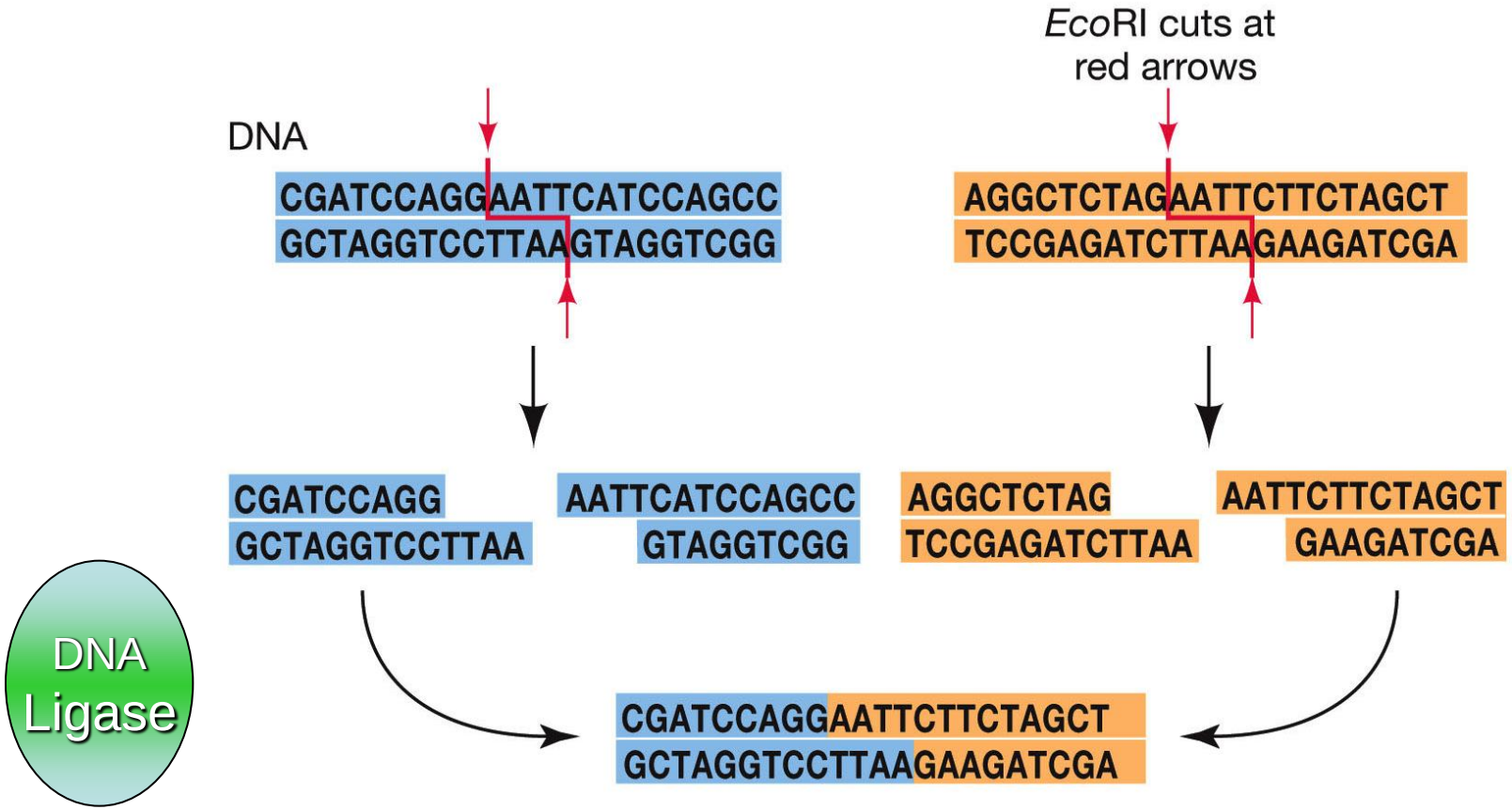
Enzyme 1: …AAGCTT… …A-3’ 5’-AGCTT…
*Hin*dIII …TTCGAA… …TTCGA-5’ 3’-A…

Enzyme 2: …CCCGGG… …CCC GGG…
*Sma*I …GGGCCC… …GGG CCC…

Restriction enzymes recogise palindromic sequences



Cut DNA fragments can be joined in new combinations

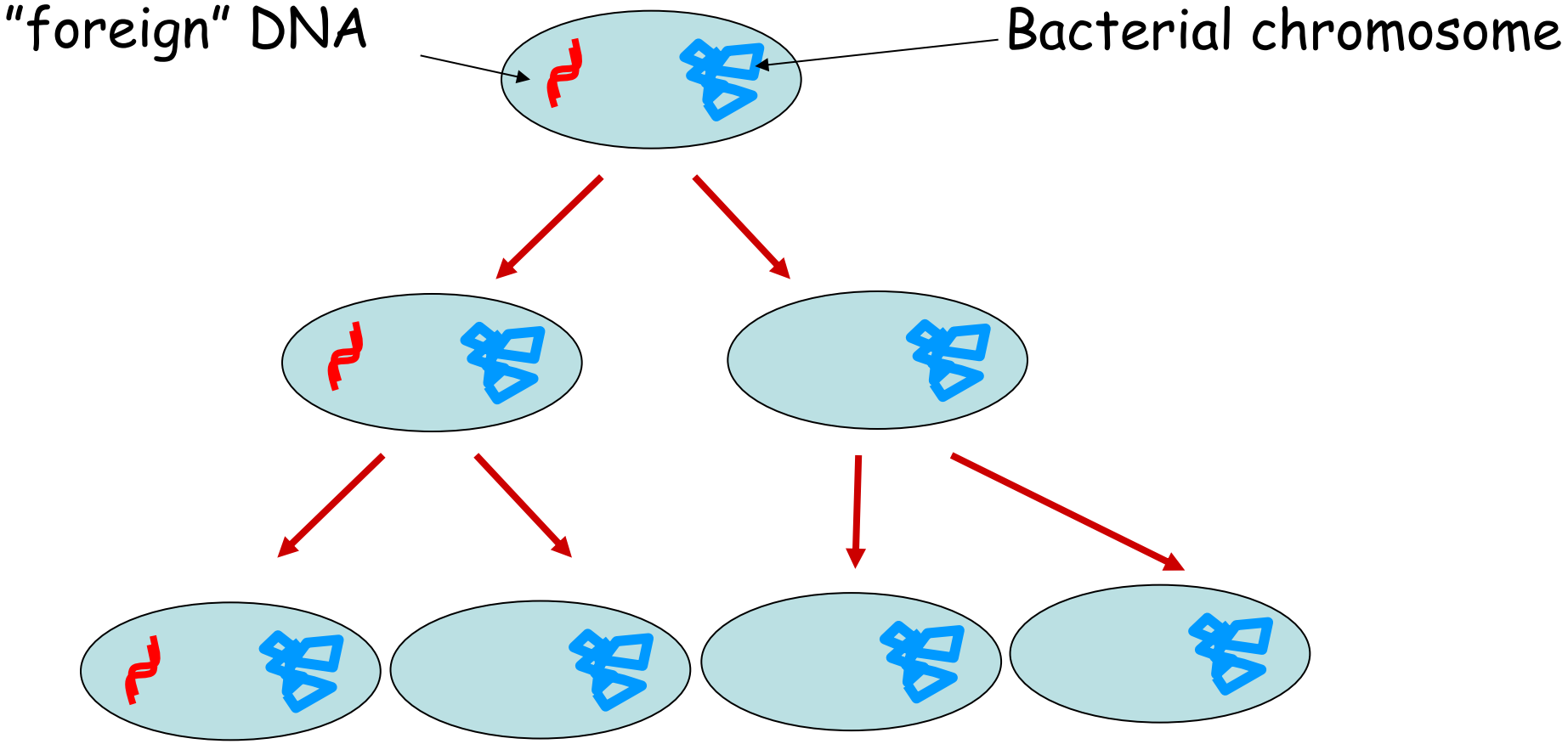


Bacteria can take up DNA molecules

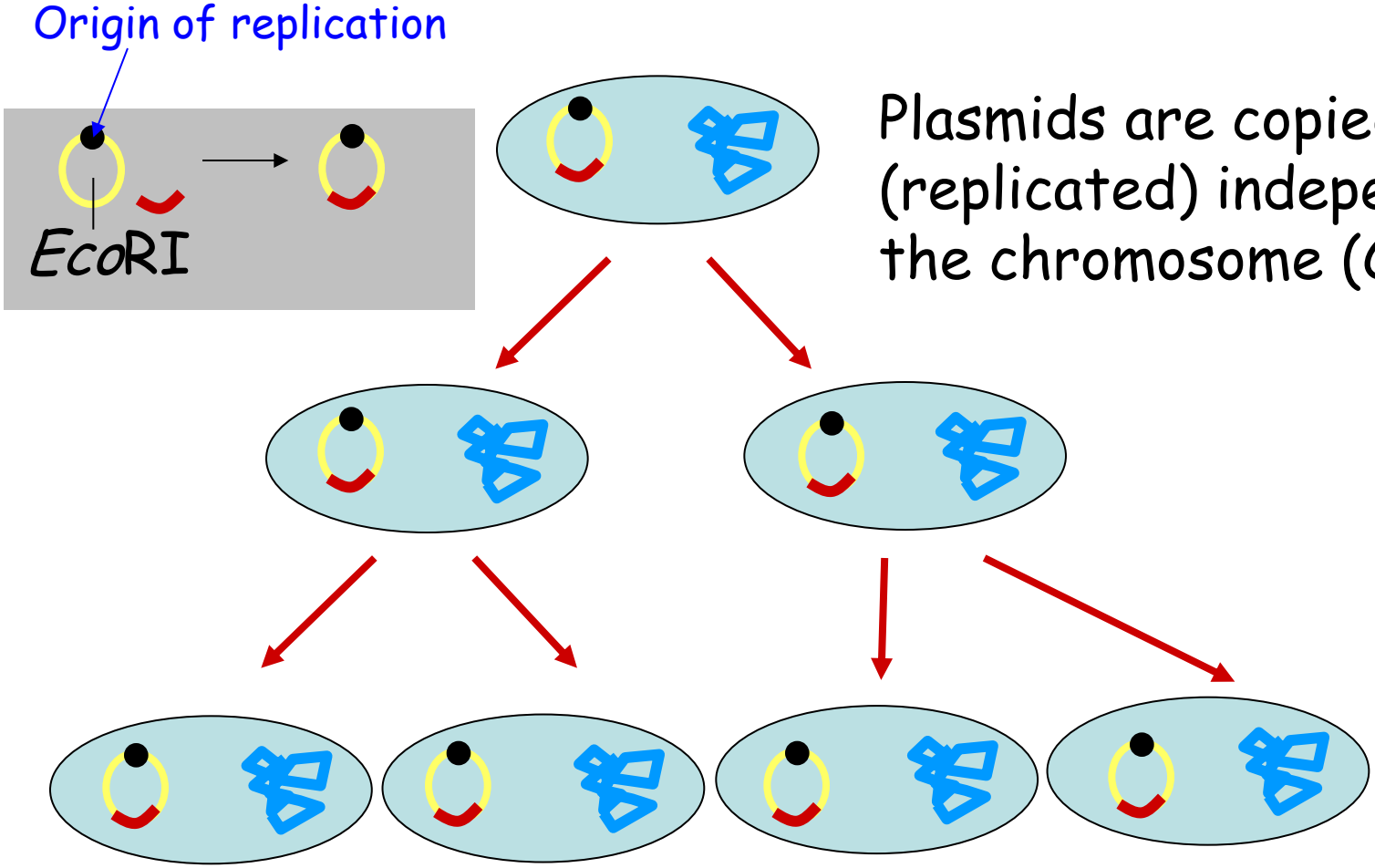
- Transformation
- Transduction
- Conjugation

- See Ch. 12,13,16

The new DNA must be replicated



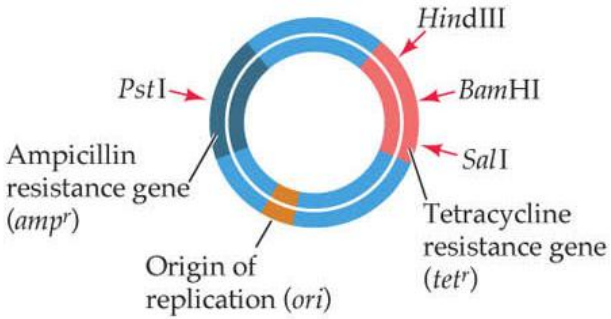
Plasmids are small circular DNA "chromosomes"



Plasmids are copied (replicated) indepedently of the chromosome (Ch 12)

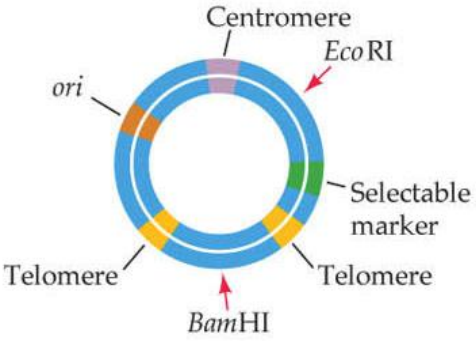
Cloning vectors

Plasmid



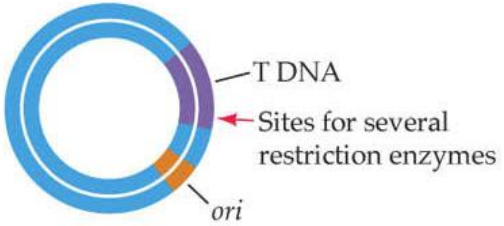
Cloing in bacteria

Yeast Artificial Chromosome (YAC)



Cloning in Yeast

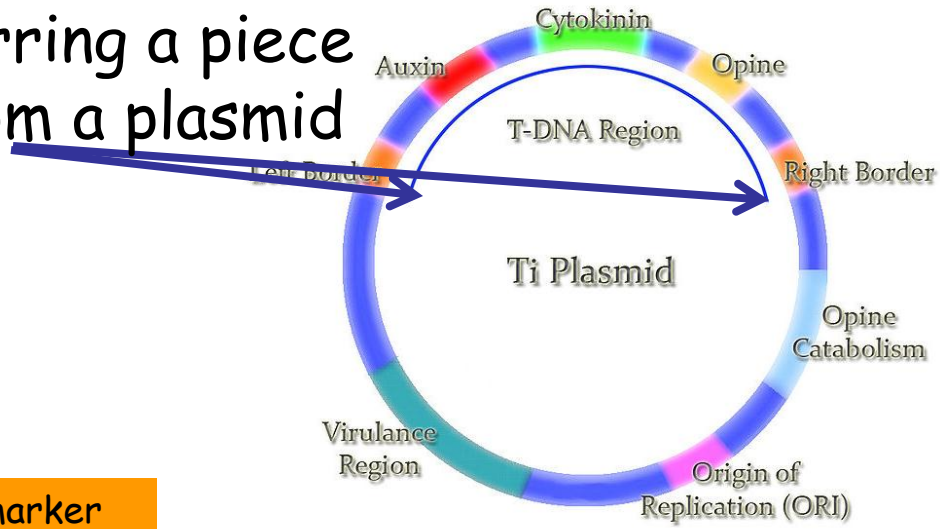
Ti Plasmid



Cloning in plants (via bacteria)

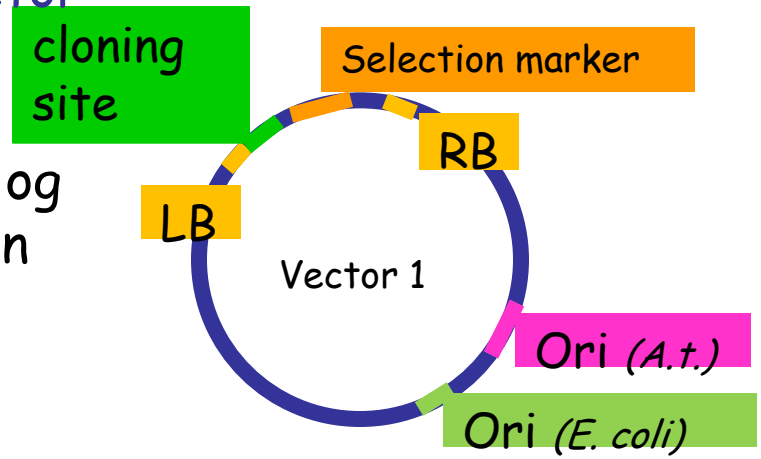
Agrobacterium tumefaciens

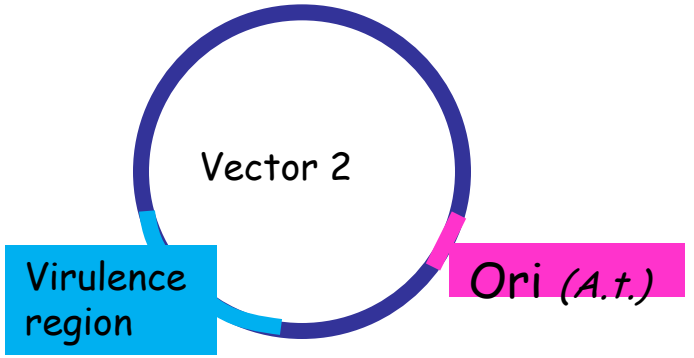
A bacterium that causes tumours in plants, by transferring a piece of DNA (T-DNA) from a plasmid to the plant genome.



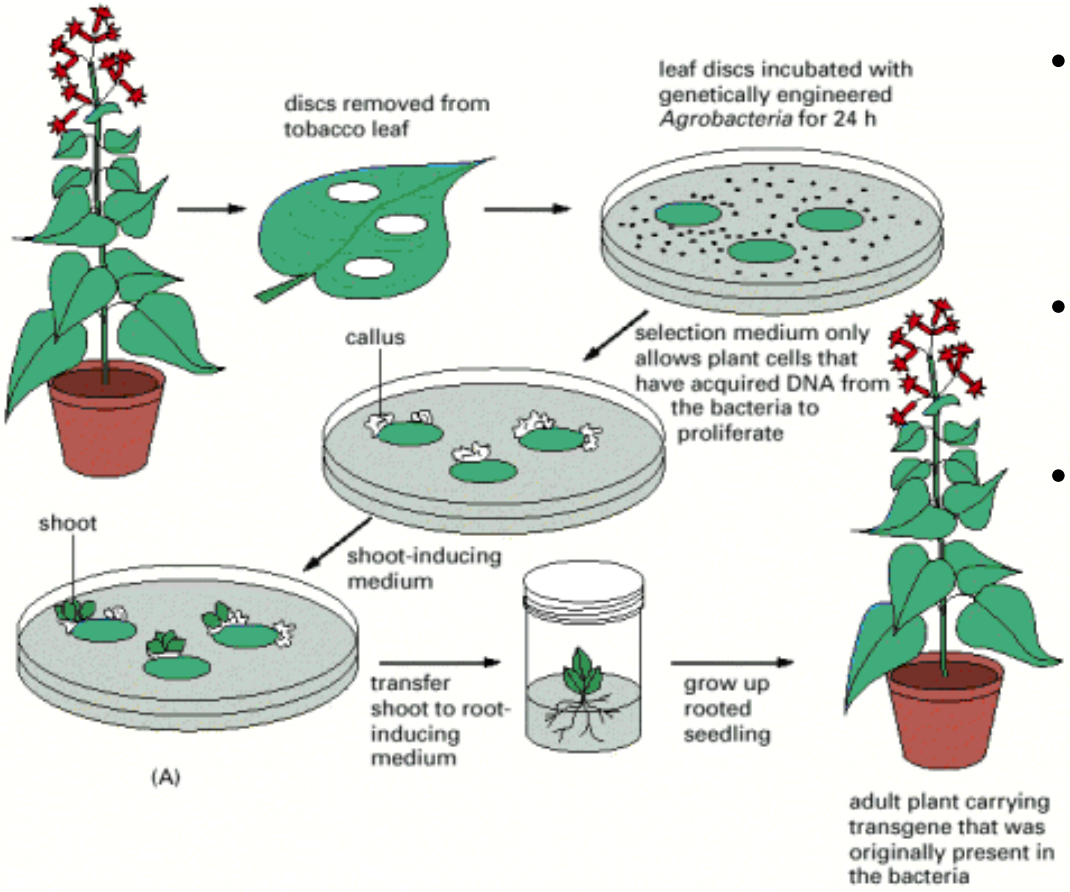
This is exploited using a binary vector system:

DNA between LB og RB is integrated in the plant genome





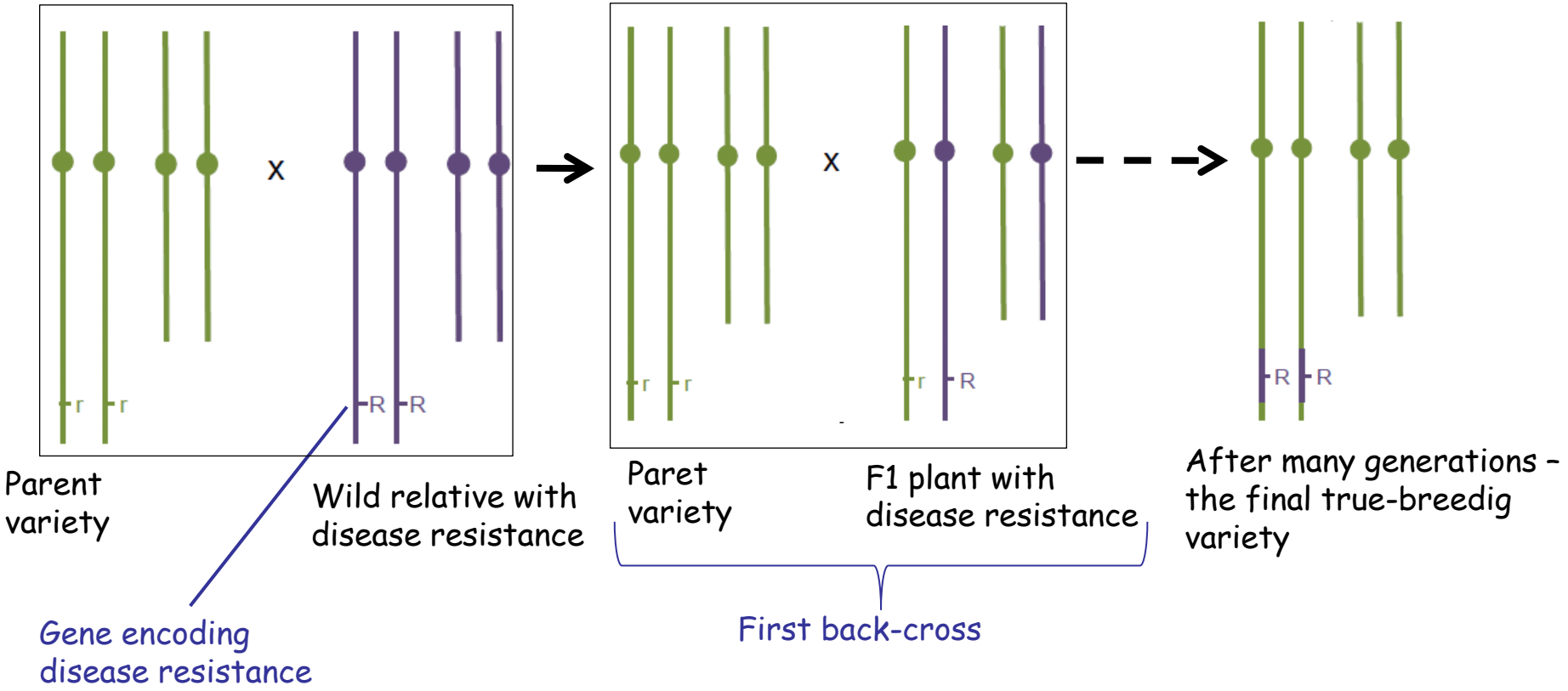
Agrobacterium tumefaciens



- *Agrobacterium* transfers DNA to plant
- Hormones induce growth of new plants
- The T-DNA also contains a selection marker

Plant breeding is time consuming

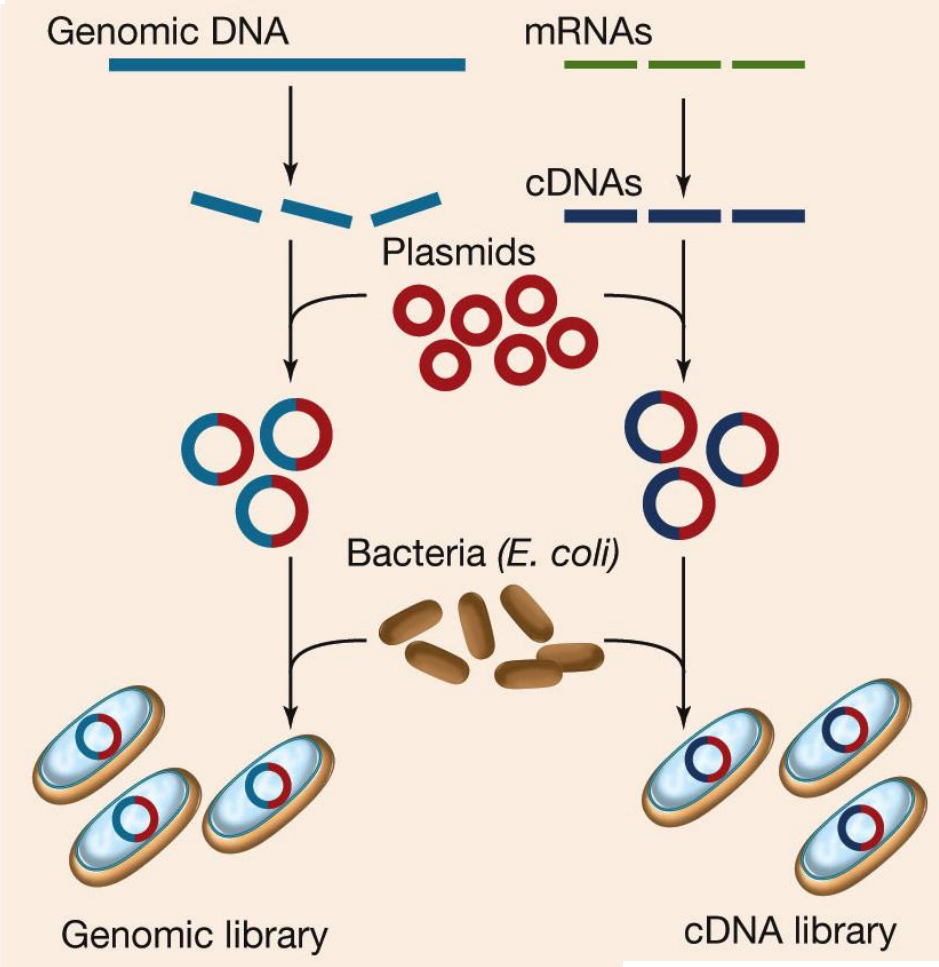
- When breeding, not just one gene is transferred



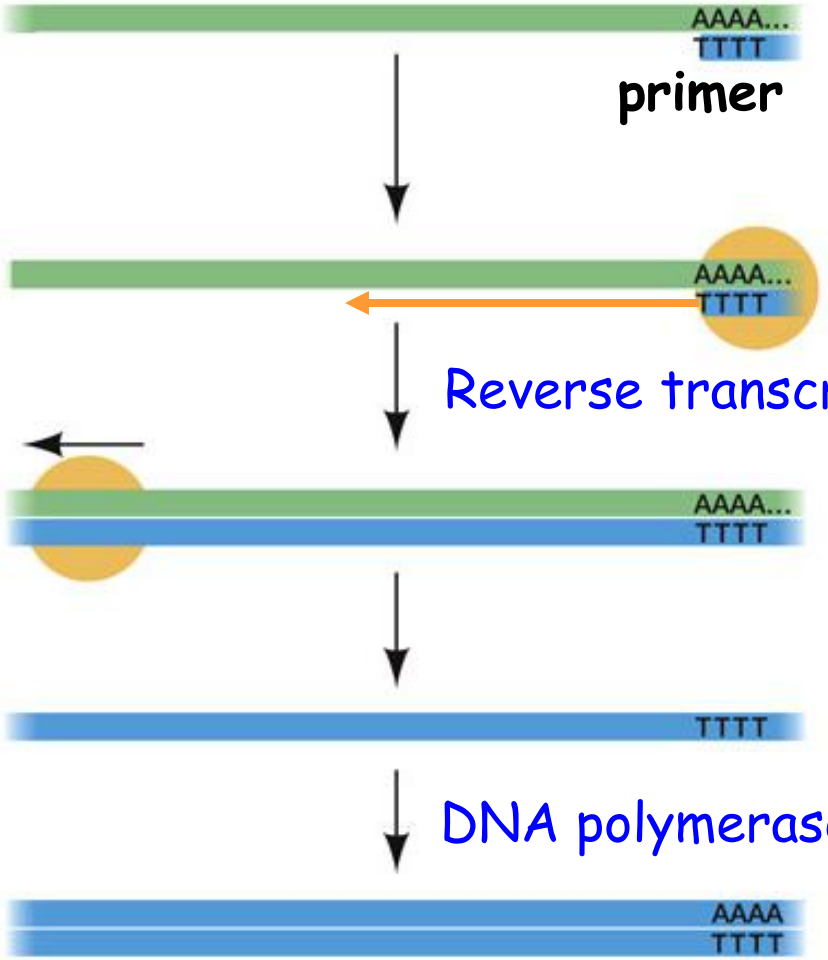
Gene libraries

- Genomic library:
  - Genomic DNA is fragmented and fragments are inserted into a vector

- cDNA library
  - Only "active" genes are cloned – those that are transcribed to mRNA



cDNA -complementary DNA



mRNA:5′-AUG CCG AUA UAA GAAAAAA-3
3′-TTTTTT-5′
Primer

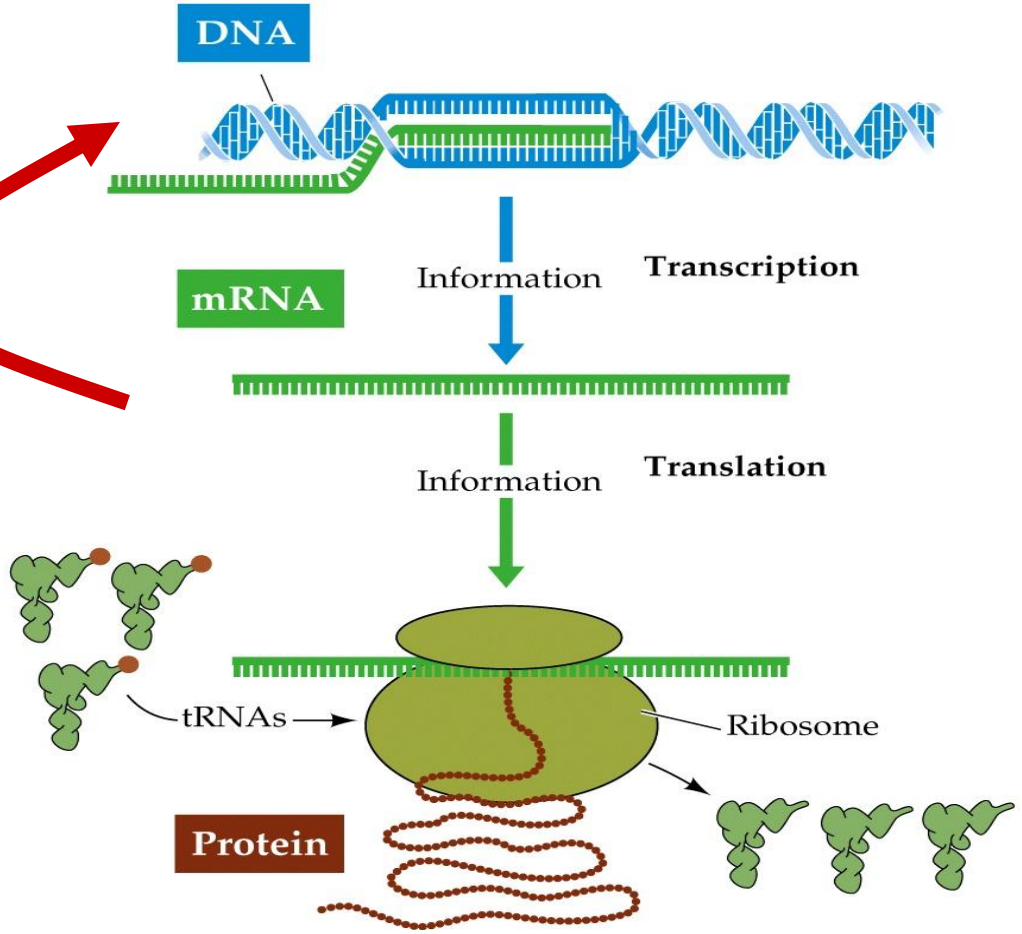
mRNA:5′-AUG CCG AUA UAA GAAAAAA-3
cDNA: 3′-TAC GGC TAT ATT CTTTTTT-5′

cDNA: 3′-TAC GGC TAT ATT CTTTTTT-5′

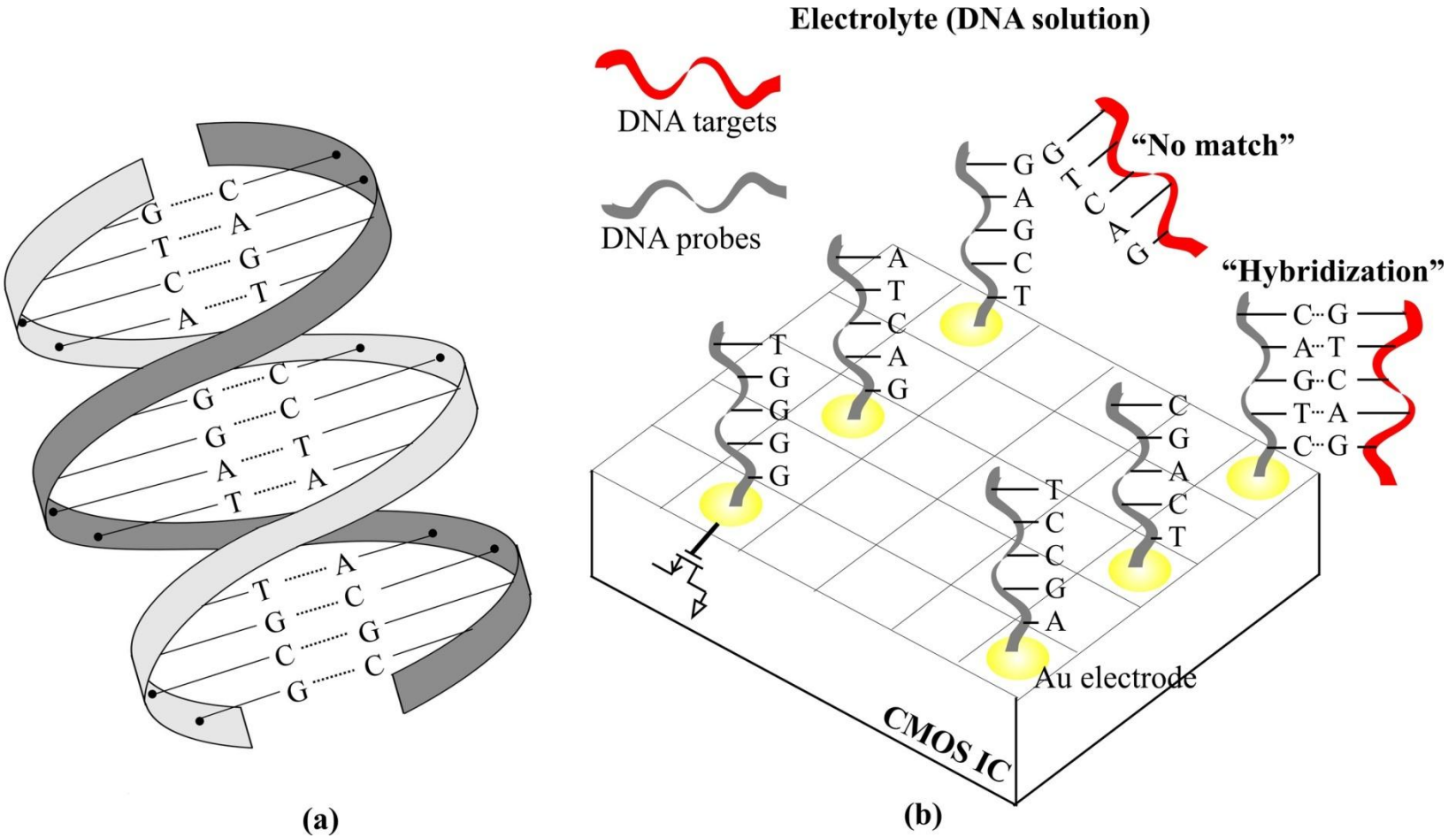
5′-ATG CCG ATA TAA GAAAAAA-3
cDNA: 3′-TAC GGC TAT ATT CTTTTTT-5′

The Central Dogma ?!

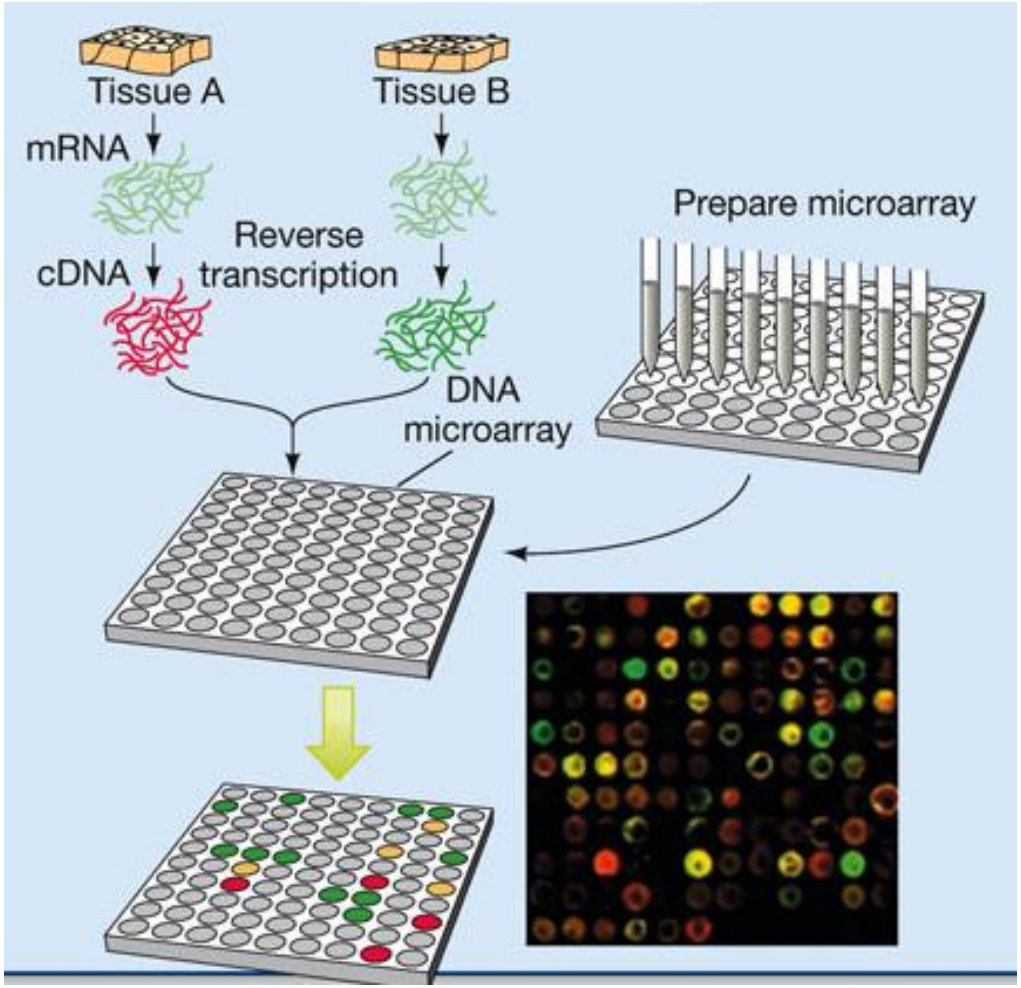
Reverse transcriptase



DNA chips- microarray



DNA chips- microarray



Which genes are expressed?

Comparison of gene expression: e.g. in healthy and diseased tissue

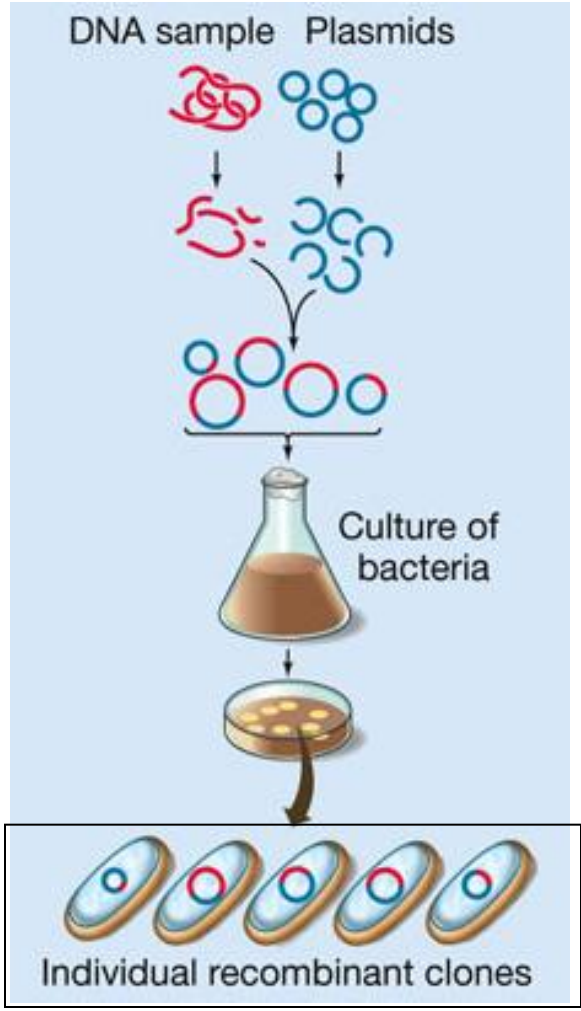
Gene libraries

- Each bacteria (or bacterial colony) contains a recombinant plasmid – with a part of the "library"

How to find the gene of interest?

-screening

-selection

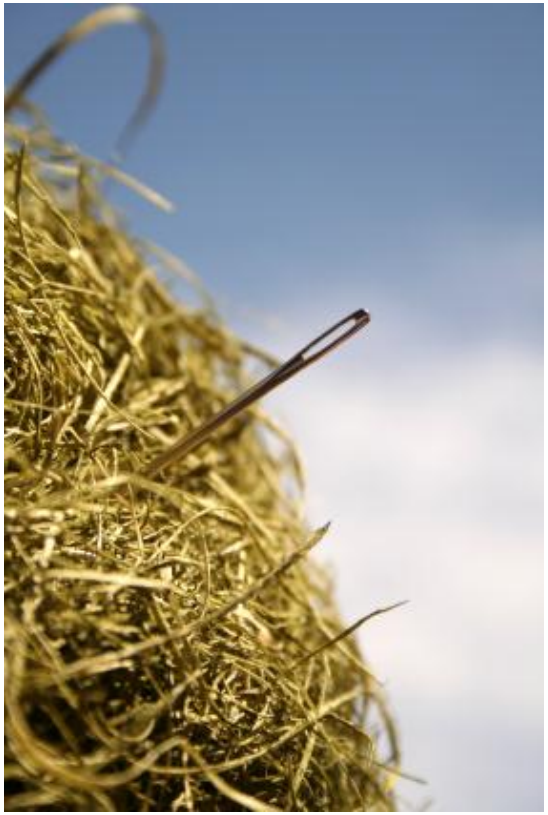


What's the difference between screening and selection?

- When screening, all candidates are examined until the "correct" one is found

- Selection exploits conditions that ensure that only the correct candidates survive

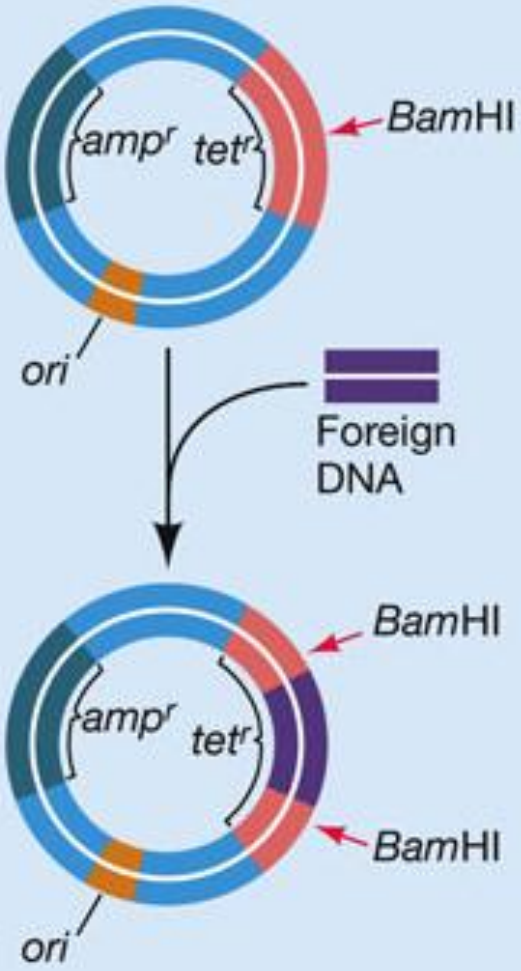
Example: "A needle in a haystack"



- Search the whole haystack
- Lie in the hay until you get pricked

- Throw the hay into a swimming pool
- Set fire to the haystack
- Use a magnet

Labelling of cells containing recombinant DNA

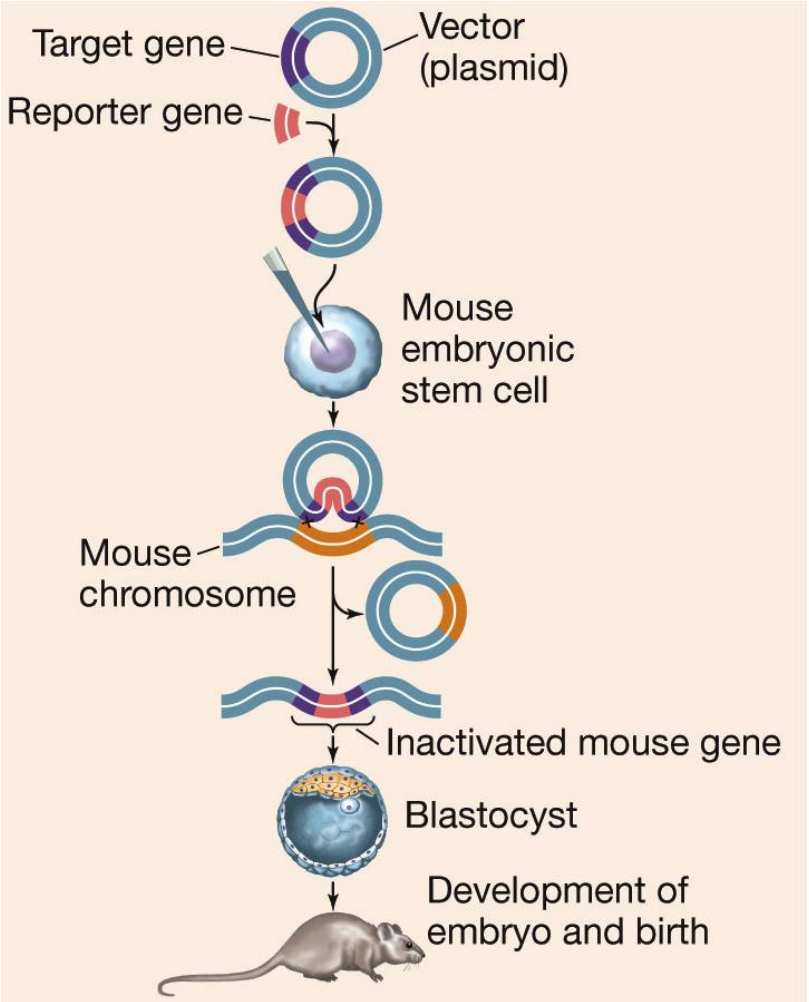


Gene knockouts

- An important tool for investigating gene function
- Transposons (Ch 17)
  - Discovered in maize by Barbara McClintock in 1940's (she was awarded the Nobel prize in 1983)
  - Later (1970's) found i other organisms
  - Transposable elements make up about 45% of the human genome
  - Can be used to screen for desired phenotypes, where the gene is not known i advance

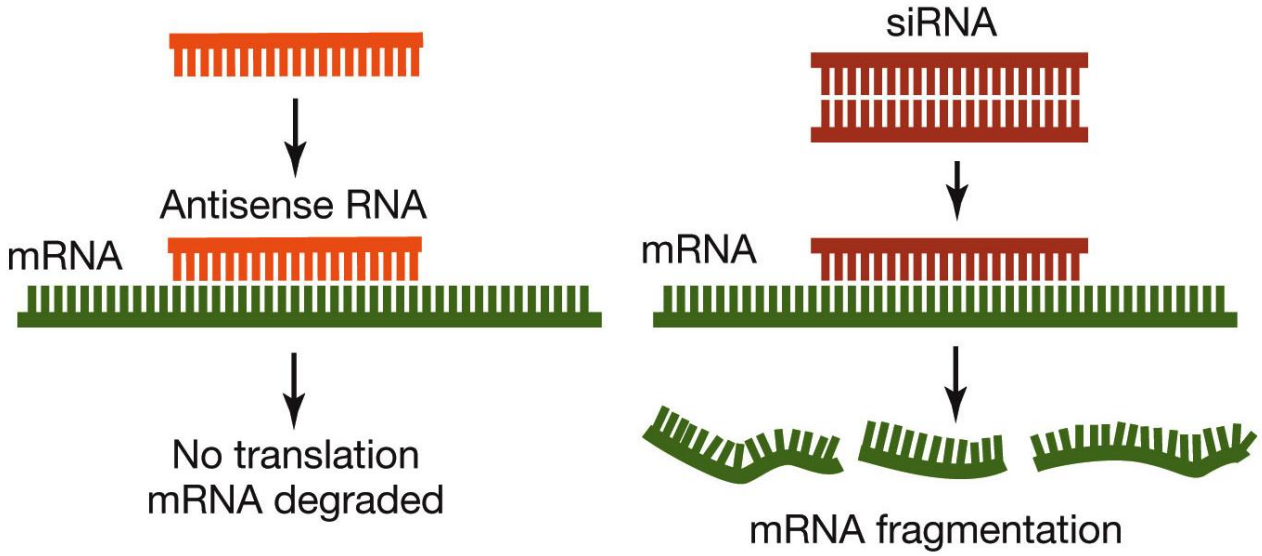
Gene Knockouts

- Homologous recombination
  - Part of the target gene is replaced by a marker gene
  - Requires knowledge of the target gene sequence you want to inactivate



Antisense og RNAi

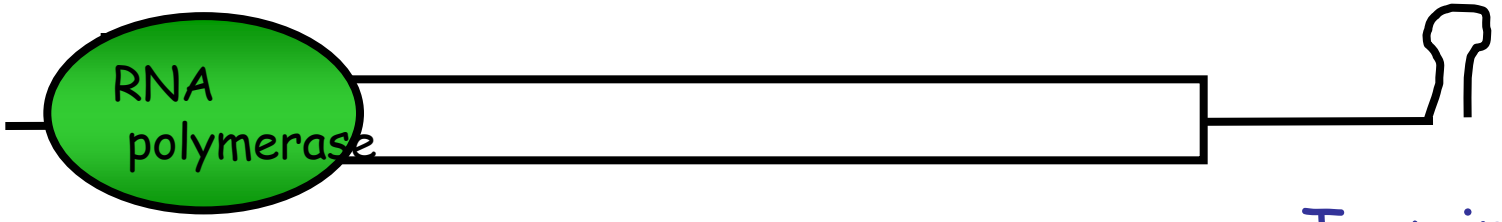
- Cells recognise double stranded RNA and degrade it
- The gene is still transcribed, but the mRNA is degraded



What is needed to express a gene i a host cell?

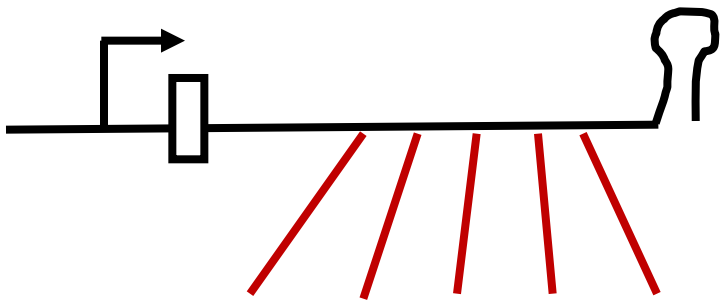
(Ch 14)

Promoter



Terminator

Ribosome binding site (Shine-Delgarno)



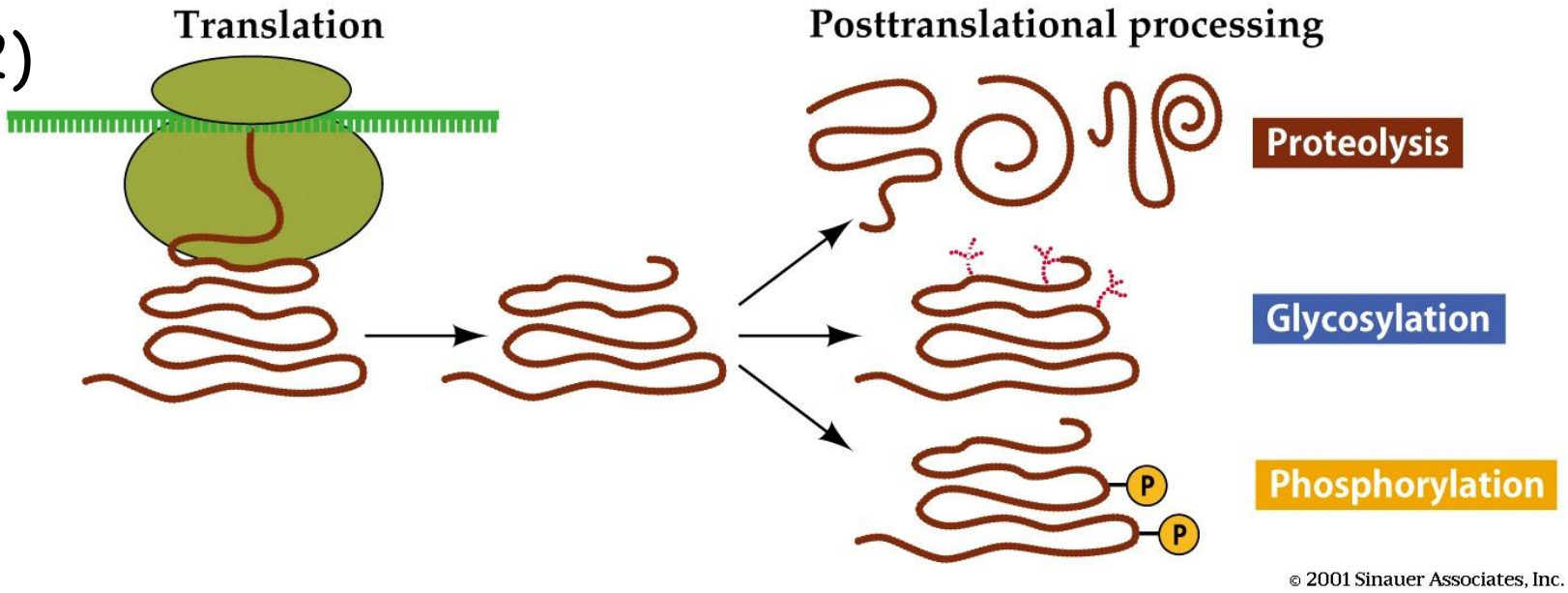
Expression vector

Multi cloning site (MCS)

*Sma*I *Eco*RI *Pst*I *Sal*I *Eco*RV

1 gene – many proteins!

(Ch 12)



Prokaryotes do not carry out the same protein modifications as eukaryotes

It is often an advantage to express eukaryotic proteins in e.g. yeast or plants, instead of bacteria

Example – molecular pharming

Production of therapeutic proteins in e.g. milk or plants